CLAIMS

1. A method for reducing total sample complexity in native or digested biological sample(s), before analysis thereof by mass spectrometry, comprising the following steps:
   a) selecting a fraction from the entire native or digested biological sample(s) on the basis of pI-value, said fraction comprising native or digested sample representing a subset of or the entire substance population in the sample;
   b) separating native or digested sample substances from each other; and
   c) analysing said substances by mass spectrometry.

2. A method according to claim 1, wherein said substances are peptides obtained from proteins in the sample(s).

3. A method according to claim 1 or 2, wherein the pI-value is 3.5 - 4.5 or a sub range thereof.

4. A method according to claim 1 or 2, wherein the pI-value is selected to target one or more specific peptides.

5. A method according to one or more of the above claims, wherein said fraction in step a) is obtained by anion exchange chromatography.

6. A method according to claim 5, wherein the separation in step b) is by cation exchange chromatography.

7. A method according to one or more of the above claims, wherein, in step a), the sample is dissolved in a buffer with pH 4.5, the sample is loaded onto an anion exchange column, and the desired peptides are eluted in a buffer with pH 3.5.

8. A method according to one or more of the above claims, wherein the separation in step b) is by multidimensional chromatography, MDLC, comprising cation exchange chromatography, RPC (reverse phase chromatography) and MS/MS.

9. A method according to one or more of the above claims, wherein the anion exchange column is coupled to the cation exchange column.

10. A method according to claims 8 or 9, wherein the pH in step a) is higher than in step b).

11. A method according to any of the claims 1-4, wherein the fraction in step a) is obtained by isoelectric focussing.

12. A method according to any of the claims 1-4, wherein the fraction in step a) is obtained by chromatofocussing.

13. A method according to claim 11 or 12, which is integrated to a conventional MDLC (multidimensional liquid chromatography) flow path.

14. A method according to one or more of the above claims, wherein the mass spectrometric analysis is tandem MS.

15. A method according to one or more of the above claims, wherein the MS is ESI (electrospray ionisation)-MS.

16. A method according to one or more of the claims 1-14, wherein the MS is MALDI (matrix assisted laser desorption ionisation)-MS.

17. A method according to one or more of the above claims, wherein the biological sample(s) comprises at least two samples which are differentially labelled.

18. A system for reducing total sample complexity in a method according to one or more of the claims 1-17, comprising a charge-selective column coupled to a MDLC work flow path comprising a cation exchange column and a RPC column.

19. A system according to claim 18, wherein the charge-selective column is an anion exchange column.

20. A system according to claim 18 or 19, wherein the charge-selective column is run with a first buffer having pH 4.5-4.0 and a second buffer having pH 3.5-4.0, wherein the second buffer has lower pH than the first buffer and is used for elution.

21. A system according to claim 18, wherein the charge-selective column is a chromatofocussing column.

22. A system according to claim 18, wherein the charge-selective column in an isoelectric focussing column.

23. A system according to one or more of the claims 20-22, wherein the cation exchange column is run with a third buffer with pH lower than the buffer used for elution from the charge-selective column.